# SOME NOTES ON THE ENCYSTED LARVA OF THE LUNG DISTOME

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In a former article (1916) I reported on (1) the discovery of the intermediate hosts (crabs) of the lung distome in Japan; (2) species of the intermediate hosts in various districts of our country; (3) the frequency of occurrence of the encysted larvae (cysts) in various crabs; (4) morphology of the encysted larva; (5) the animals, experimentally fed with the cysts, etc. The report to be given in the following pages is a part of the results obtained by subsequent study on the cysts of the lung distome in crabs, especially *Eriocheir japonicus* (de Haan).

## DISTRIBUTION AND MIGRATION OF CYSTS IN THE BODY OF AN INTERMEDIATE HOST

The encysted larvae are found in various parts of *E. japonicus,* namely, muscles, hypodermis, gills, liver and other organs; of these, muscles and gills are most abundantly infected. The absolute number of cysts is greater in the muscles than in the gills, but the relative number is inverse, because the volume of the muscles is much larger than that of the gills. The cysts in the gills are found only along a limited portion of blood vessels running longitudinally on the median line of their upper surfaces. In the muscles the cysts are found most frequently and most abundantly in the base of each appendage. Numerous cysts are often found in the muscles near the basipodite of each appendage, even in the cases in which a few or none of cysts are found in other parts of the musculature.

From the abundance of the cysts in gills and near their attachment, the basipodite of each leg, and from the system of blood circulation in the crab, I am inclined to believe that the encysted larvae have a tendency to migrate toward the gills from all parts of body by means of the blood circulation. It was experimentally proved that the cyst has the ability to migrate through the various tissues of the crab, although the rate of migration is very slow. On the other hand, the circulatory system of the crab is open, as the distal ends of the arteries open into the tissues of the body and thus all tissues are bathed in the blood. The venous system begins not with capillaries, as in a closed system, but with lacunae, lying irregularly among the tissues. The lacunar spaces in the tissues communicate with one another at first, and gradually form a canal system after union of several lacunae from different parts; ultimately these grow into the venous vessels which run toward the gills to purify the blood. The blood current among the tissues and in the vessels of the venous system surely facilitates the migration of the cysts toward the gills. If this supposition is right, it explains clearly why the cysts are found abundantly in the small blood vessels in the gills and in the muscle near the base of each appendage.

Thus the venous vessel is the most convenient course by which the cysts migrate toward the gills. For what purpose do the cysts migrate to the gills? Is there any necessity for the cysts to migrate to the gills? It is most favorable, I believe, for the cysts to migrate to the gills in order to facilitate further development of the encysted larvae by getting into the final host. On the whole, there are two ways by which the cysts may be taken up by the final hosts—human beings or other animals as dog, cat, etc.—namely: (1) their consumption as a food in an uncooked crab; (2) being taken with food and drink infected with cysts liberated into water from the intermediate host. In the second method of infection it is necessary for the cysts to escape into the water from the infected crab. The gills are the most convenient point at which the cysts can escape easily into the water, because the organ is always being laved by water and the blood vessel containing the cysts is separated from the outside water only by the very thin membranous wall. Thus it is reasonable to think that the cysts in various parts of the intermediate host migrate through the tissues carried by the blood current in the venous vessels toward the gills from which they may be discharged into the water.

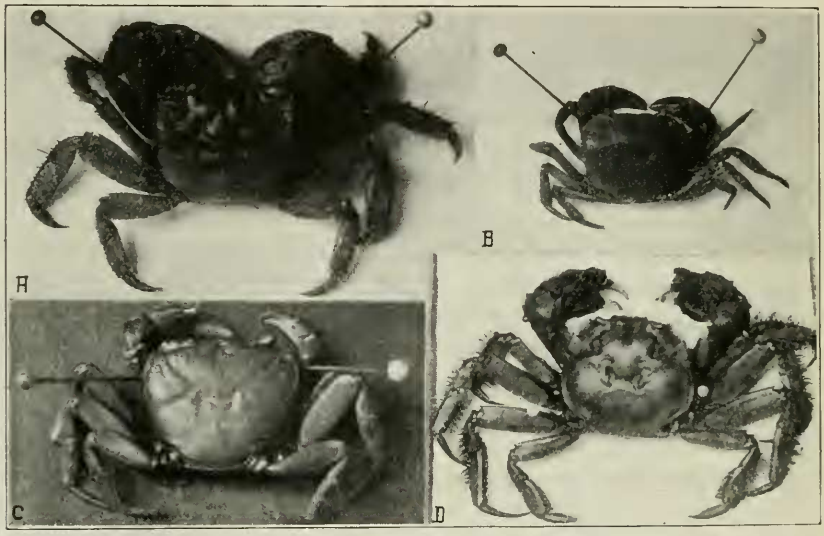
It is questionable in my mind whether the cysts in a crab (*E. japonicus*) escape into the water naturally and actively to secure an opportunity of being taken up by the final host. In Corea, R. Moriyasu, E. Arima, and M. Tanaka proved experimentally the natural and active escape of cysts in the case of *E. japonicus*. In Formosa, K. Nakagawa obtained the same results as Moriyasu in the case of *P. obtusipes* (Stimpson). In Japan proper, R. Ando also proved experimentally that the result is quite the same in the case of *P. dehaanii* (White). All these writers made their experiments by approximately similar methods, namely, putting ten to thirty specimens of a crab which seemed to be infected with the cysts into cylindrical glass vessels with a little water. Renewing the water once or twice a day, they searched for cysts. In these examinations they all found the cysts more or less numerous, and hence concluded that the cysts escaped naturally and actively from the body of crab. In the case of *P. dehaanii* and *P. obtusipes,* it is possible that the cysts in the crab may escape into the water naturally and actively, because in these intermediate hosts the cysts are often found attached to the outer surface of gills, as I reported in the former paper (1916).

I have also made some experiments to prove the natural and active discharge of cysts from the crab. There were two sets of experiments: 1. I prepared a glass aquarium 75 cm. long, 27 cm. wide and 29 cm. deep, provided with three small exits on the bottom, the upper side being open and covered by metal gauze when necessary. Twenty crabs or more of moderate size were put into the aquarium, and water was permitted to flow in by a pipe and out through three exits which were closed by two or three sheets of gauze in order to prevent the escape of the cysts. I examined the sediment on the bottom occasionally for cysts and have often found them among materials there. 2. I put one or two specimens of crab into a cylindrical glass vessel 18 cm. in diameter and 15 cm. deep, pouring in water to a depth of 3 cm. or more. I prepared five such vessels, and renewed the water once or twice a day. This set of experiments was continued three months, having been started October 4 last year. In this long-continued experiment, only one cyst came under my observation. In these experiments I used the crabs *E. japonicus* (de Haan) from Tomioka, *Tokushima Prefecture,* which were abundantly and frequently infected with the cysts of the lung distome.

In the first set of experiments I frequently found numerous cysts among other sediment. I have occasionally found dead crabs in the aquarium and pieces of legs and other parts of the body were always present in the material on the bottom of the vessel. It is reasonable to think that the cysts in the crab are easily discharged from the body when the crab is dead or any part of body is accidentally injured. Hence from observation of the above facts I believe that these free cysts in the aquarium were unnaturally and passively discharged from the body by occasion of death or some injury.

When the crabs used in the second set of experiments were dead the substitute crabs were usually transported from the aquarium of the first set. It is even possible that the one cyst which I found in the second was not naturally and actively discharged from body of crab itself, but was carried attached to some part of the body from the aquarium in which I had proved the presence of cysts as stated above. The thickly haired forceps of the crab may be a good carrier of cysts, adhering to it even in case the crab had been carefully washed to remove attached particles.

In considering the facts observed in the above two sets of experiments one may say that if the cysts in the first aquarium had been naturally and actively discharged from the crabs I should have found more numerous cysts in the vessels of the second series than were found actually. But in reality, there was only one cyst in the vessels of the first set during a long time. Thus I conclude from my own experiments that cysts in the intermediate host (*E. japonicus*) are not naturally and actively discharged from the body, but are often expelled unnaturally and passively by death of the crabs or some injury. In nature there are many occasions favorable for cysts escaping from crabs unnaturally and passively, namely, death of the crabs, frequent injuries by the fierce quarrels of the warlike crabs, breaking legs in slight disturbances, and accidental injuries in the period of moulting, etc.




Japanse River Crabs which serve as intermediate hosts for *Paragonimus westermanii*. A. *Sesarma dehaani* M. Edwards. B. *Potamon dehaanii* (White). C. *Potamon obtusipes* (Stimpson). D. *Eriocheir japonicus* (de Haan). Photographs by Mr. Koyama.

LONGEVITY OF CYST IN WATER

For studying the transfer of this cyst to a final host, it is most important to know how many days the cyst can be kept alive naturally in water. I have made the following experiments to determine this matter: To keep the cysts in water in a state as similar to natural conditions as possible, I prepared a small glass aquarium of 30 cm. long, 20 cm. wide and 17 cm. deep with the bottom provided with one small exit. Water was constantly pouring into it by the inflow pipe and flowing out through the exit on the bottom, so the water in the aquarium was always moving and being renewed as in a running stream. For convenience in examining perfectly changes in the cysts and counting accurately the number of cysts dead or alive, I used as a case for holding them a glass tube opening at both ends covered by one or two sheets of gauze and filter paper to prevent the cysts escaping from the tube but to permit the water to flow in and out though not freely.

(A) I put forty-two cysts from the gills of *E. japonicus* in a tube whose ends were closed by gauze. The tube was placed in the aquarium October 30 and taken out for examination November 12, having been in water thirteen days.

(B) Twenty-five cysts from the gills of the same species of crab were put in a tube, one end of which was closed by layers of gauze and two layers of filter paper and the other end by two layers of gauze and one layer of filter paper. The tube was kept in the aquarium from November 12 to 27, an interval of fifteen days.

(C) Twenty-five cysts from the gills and muscles of the same species of crab were put in a tube whose ends were closed by two layers of gauze and two layers of filter paper. The tube was left in the aquarium from November 12 to December 10, or twenty-eight days.

(D) November 15 I removed the cysts with surrounding tissues of the host from the gills and muscles of a specimen of *E. japonicus* that had died November 12. Twenty of these cysts were put in a tube whose ends were closed as in Case C. The tube was immersed in the aquarium during twenty-five days from November 15 to December 10.

The results of these experiments are listed as follows:

| Case | Total Number | Living One | Dead One | Cyst Only | Percentage | Days |
|---|---|---|---|---|---|---|
| A | 26 | 10 (in cyst)<br>4 (outside) | 4 (in cyst)<br>1 (outside) | 4 | 53.8 | 13 |
| B | 25 | 4 (in cyst)<br>1 (outside) | 5 (in cyst)<br>1 (outside) | 14 | 20.0 | 15 |
| C | 25 | (All were dead and decomposed) | | | .... | 28 |
| D | 20 | 2 | 2 | 14 | 10.0 | 25 |

In Case A twenty-six out of forty-two cysts were found in the tube and the remaining were lost. The loss may be perhaps due to having closed both ends of the tube with gauze only. To avoid this defect in Cases B, C and D, I had used both gauze and filter paper for closing the tube ends, the latter being placed inside of the former.

Cysts containing living larva were not all perfect, some of them being slightly broken and the others so widely broken that the larva was creeping out. I found there were living worms also in various stages. Some of them were actively moving with the light red pigment in the body as observed in fresh larvae, others moved slowly, and some others appeared dead, having no apparent motion. In the last group the light red pigment was greatly reduced or entirely absent. Various gradations of morphological change and putrefaction were observed in dead worms. In almost all the cysts, whether the worm was alive or dead, swarmed an immense number of flagellata of various species.

From my experiments above it is evident that the encysted larva may be kept alive relatively long in water. If a larger tube be used instead of a small one as in my experiments and both ends of the tube be closed by other suitable materials which make the circulation of water in the tube as perfect as possible under the conditions that retain the cysts, putrefaction of the cysts and their surrounding host tissues would be delayed and consequently the cysts would remain alive for longer time. Therefore we may conclude that cysts in water remain alive at least for thirty days under natural conditions.

From my other experiments it is known that cysts in the crab may be kept alive for a week in the winter season, the gills and other inner parts of the crab being exposed to an air by taking off the carapace.

## METHOD OF INFECTION

There are two ways in which the human host may be infected with encysted larvae from the crab: (1) by taking as food an uncooked crab infected with living cysts; (2) by taking with food and drink living cysts discharged from the crabs. Which of these two ways of infection is common may be quite different in various districts of the country, varying according to the species of intermediate host and to the custom of people in the district. One intermediate host, *E. japonicus,* is edible and used as food in all districts of Japan, but it is generally eaten cooked—boiled, roasted or fried—and is rarely used uncooked. Another crab, *P. dehaanii,* is also edible and eaten cooked or uncooked in general. In some districts it is customary to use it uncooked in certain season of year. People in these districts are easily and commonly infected by eating uncooked crabs and a large percentage of those people are found to be afflicted with lung distomiasis. *S. dehaani* is not taken as food and human infection will be brought about by the second method in the districts where *S. dehaani* happens to be the only intermediate host present.

For prophylaxis in the disease caused by the lung distome the following are necessary conditions: Not partaking of uncooked crabs and other foods washed in water in an infected district. Not drinking unboiled water in such district.

## Reference Cited

Yoshida, S. 1916. On the Intermediate Hosts of the Lung Distome, *P. westermanii* Kerbert. Jour. Parasitol., 2: 111-118; 1 pl.